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Original Research Article

Evaluation of the Antioxidant, Anxiolytic and Anti-Epileptogenic Effects of *Vitex chrysocarpa* Leaf

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ABSTRACT

Vitex chrysocarpa is a medicinal plant widely used in traditional medicine across the native parts of tropical Africa for treatment of various health conditions including anxiety, epilepsy, oxidative stress, microbial infections and inflammation. This study aimed to investigate the antioxidant, anxiolytic and anti-epileptogenic activities of *Vitex chrysocarpa* leaf methanol extract using established models. The 2,2, azino-bis(3ethylbenzthiazoline-6-sulphonic acid) (ABTS) free radical scavenging activity, total flavonoid, total phenolic ferric reducing antioxidant power and total capacity assays was used to measure the antioxidant activity, while Lorke's method was used to evaluate the oral acute toxicity (LD_{50}) of the extract in mice. Elevated plus maze model was used to evaluate the anxiolytic activity while pentyleneterazole-induced seizure model was used to evaluate the anti-epileptogenic activity of the extract in mice. In the anxiolytic and anti-epileptogenic studies, graded doses of the extract (200 – 800 mg/kg p.o) were evaluated, while diazepam was served as positive control. Results from the study revealed that the LD_{50} of the extract in mice was greater than 5000 mg/kg. The extract demonstrated good antioxidant activity having 47.28% inhibition at 400 µg/ mL in the ABTS assay. The extract also had significant ($p < 0.05$) anxiolytic and anti-epileptogenic effects during the study. The study outcome thus shows that *Vitex Chrysocarpa* leaf extract had potent antioxidant, anxiolytic and anti-epileptogenic activities hence may serve as scientific justification for its use in ethnomedicine.

Keywords: Antioxidant, Anxiolytic, Anti-epileptogenic, *Vitex chrysocarpa*, 2,2, Azino-bis(3ethylbenzthiazoline-6-sulphonic acid)

Introduction

Central nervous system (CNS) disorders are known to impede the structural and functional integrity of the central nervous system. [1] Chronic anxiety a common CNS disorder is often associated with increased oxidative stress. Brain areas involved in anxiety, such as the amygdala and hippocampus, are highly sensitive to oxidative damage. Oxidative stress is implicated in numerous chronic and degenerative diseases, including cardiovascular diseases, neurodegenerative disorders, and cancer. Reports show that over 301 million people are affected globally by anxiety disorders. [1] The 2021 global prevalence rate of anxiety disorder among individuals aged 10-24 years was 0.28 %, which has significantly increased in recent years, particularly during the onset of the COVID-19 pandemic, making it one of the most common health conditions. [2] Anxiety is a psychological phenomenon that results in harmful emotional experiences. It is an integral component of the normal human response to potential danger or threat and is characterized by elevated arousal, expectancy, as well as neuroendocrine and autonomic activation, accompanied by specific behavioral patterns. [3]

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Epilepsy is a chronic neurological disorder characterized by persistent, unprovoked seizures, which are usually accompanied by partial or total loss of consciousness. Its etiology could be hereditary or caused by head injury and developmental disorders (e.g., autism). [4] Globally, about 50 million people are affected by epilepsy, with the point prevalence of active epilepsy being about 6.38 per 1,000 persons, equating to 0.63 8% of the global population.[5] Epilepsy is a serious neurological condition associated with stigma, psychiatric comorbidity, and high economic cost. Seizures cause increased production of reactive oxygen species (ROS) in the brain, and the oxidative damage can worsen neuronal injury and promote more seizures, creating a vicious cycle. [6] Antioxidants help protect neurons by neutralizing ROS, reducing seizure severity and frequency. Some studies have shown that antioxidant-rich agents can have antiepileptogenic effects. [7] Though several drug classes are currently available for the management of anxiety and epilepsy, different adverse effects and tolerance characterize the use of most of these agents with prolonged use. This thus represents important research aimed at developing newer agents with fewer adverse effects. Medicinal plants are known to have therapeutic effects against various ailments, including neurological disorders, hence creating a better understanding for medical practitioners in effective treatment for various neurological issues. [8] Among the medicinal plants used in Nigerian traditional medicine is *Vitex chrysocarpa*, rooted in its traditional medicine practices and its pharmacologically active ingredients. *Vitex chrysocarpa* has been used in traditional medicine for several purposes, including treatment of hormonal imbalance, fever reduction, several skin conditions, anti-inflammation, pain and central nervous system disorders. [9] However, there remains a gap in knowledge regarding the scientific basis for its ethnomedicinal application and also there is a need for studies on its therapeutic

potentials. Hence, this study aimed to evaluate the anxiolytic, antioxidant, and antiepileptogenic activities of *Vitex chrysocarpa* methanol extract.

Materials and Methods

Plant collection and identification

Fresh leaves of *Vitex chrysocarpa* were collected November, 2023 from a botanical garden in Jos-North Local Government Area, Plateau state, Nigeria. It was identified by Mr. Jeffrey Azila, a taxonomist at the Federal College of Forestry, Jos, Plateau state, Nigeria.

Extraction

The plant leaves were air-dried for two weeks (at room temperature) and pulverized using an electric motorized plant grinder. The pulverized plant material (2 kg) was macerated in 60 % methanol (10 L) for 48 h at room temperature using standard procedures previously described by Handa *et al*. [10] The resultant micelle solution was filtered using a mesh sieve, cotton plugged funnel and Whatman filter paper No. 1. The collected filtrate was concentrated at 40°C using a water bath.

Phytochemical analysis

Qualitative phytochemical analysis was carried-out to identify the bioactive constituents of *V. chrysocarpa* extract. Tests for various phytochemicals including alkaloids, saponins, tannins, flavonoids and phenols were carried out using standard methods described by Trease and Evans.[11]

Drugs and chemicals

The following drugs and chemicals were used: Pentalenetetrazone (SIGMA chemical company, United States); isoniazid (SIGMA chemical company, United States); diazepam (Swiss parenterals Ltd, India); methanol (GHTECH, China). All drugs/chemicals used were freshly prepared daily from original solution before usage.

Antioxidant studies

2,2'-azino-bis(3ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

ABTS radical-scavenging activity was determined according to the method described by Re *et al*. [12] The ABTS radical positive charged ion was formed following the reaction of ABTS stock solution (5 mL) and 2.45 mM potassium persulphate ($K_2S_2O_8$) solution (5 mL), then stored in a dark room at atmospheric temperature for 16 hours. The extract (0.5 ml) was added to 4.5 ml ABTS radical positive charged ion solution in test tubes, then allowed to incubate at atmospheric temperature for 6 min. Absorbance was taken at 734 nm. Blanks were also done in each assay. The inhibition percentage was calculated using the following formula:

$$\text{ABTS scavenging activity} = \frac{A0 - A1}{A0} \times 100$$

A0 = absorbance of the control
A1 = absorbance of the extract.

Total flavonoid assay

Total flavonoid content of the extract was determined with colourimetricaluminium chloride methods as described by Ebrahimzadeh *et al.* [13] Five milliliter's (5 ml) of 2% aluminum (III) chloride ($AlCl_3$) in methanol was mixed with 5 ml of the extract. Absorbance was read at 415 nm after 10 min against a blank sample consisting of a 5 ml extract solution with 5 ml methanol without $AlCl_3$.Total flavonoid content of the extract was calculated using a standard curve with rutin (0 - 100 mg/l) as the standard.

Total phenolic content

Determination of total phenolic content of the extract was carried out according to the method described by Dewanto *et al.* [14] The extract (0.5 mg) was dissolved in Folin-Ciocalteau reagent (100 µl) and distilled water (6 ml). The mixture was vortexed for 1 minute, and 2 ml of 15% Na_2CO_3 was added and vortexed once again for 30 seconds. The solution was made up with distilled water to 10 ml. After 1 hour 30 minutes, absorbance was read at 750 nm with a UV spectrophotometer. Gallic acid solution was used for preparation of the calibration curve. Total phenolic content of the extract expressed as milligrams of gallic acid equivalent (mg GAE)/100 g of dry weight

Ferric reducing antioxidant power (FRAP) assay

The ability ferric reducing antioxidant power was measured using the method described by Benzie and Strain. [15] FRAP reagent was prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM TPTZ (tripyridyltriazine) solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Different concentrations of the extract (100 – 400µg/ml) (0.5 ml) were added to the FRAP reagent (3 ml). The reaction mixture was raised at 37°C for 30 min. The increase in absorbance was measured at 593 nm using a spectrophotometer. Ferric reducing antioxidant power was calculated using the following formular:

$$\text{FRAP} = \frac{A1 - A2}{A1} \times 100$$

A1 = absorbance of the control,
A2 = absorbance of the extract.

Total antioxidant capacity (TAC)

Total antioxidant capacity in the extract was evaluated using the method described by Prieto *et al.* [16] The extract (0.1 mL) was added to 1 mL of the reagent (28 mmol/L Na_3PO_4, 4 mmol/L ammonium molybdate and 0.6 mol/L H_2SO_4) in test tubes. The tubes were raised in a thermal block at 95 ºC for 90 min. The combination was allowed to cool at room temperature. Absorbance was measured at 695 nm against blank. Antioxidant capacity remained stated as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 0 – 500 mg/mL.

Experimental animals

Swiss albino mice of both sexes housed at the animal facility of the Department of Pharmacology, Novena University were used. The animals were housed in standard cages under standard laboratory conditions in accordance with the "NIH guidelines for laboratory animal care and use" (National Research Council, 1985) [17] and the Novena University regulations for laboratory animal use.

Oral acute toxicity test (LD_{50})

The acute oral toxicity of the *V. chrysocarpa* extract was carried-out on Swiss albino mice using Lorke's method as described by Enegide *et al*. [18 - 19] A total of 12 mice of either sex were fasted overnight prior to the study. The study involved two phases. In the first phase, nine mice were randomly divided into three groups of three mice each and were administered extract doses of 10, 100 and 1000 mg/kg p.o respectively. In the second phase, three fresh mice were divided into three groups, one per group. Doses were selected based on the lethality in in the first phase. Extract doses of 1600, 2900 and 5000 mg/kg p.o were administered to the respective groups. The animals used for both phases were observed for signs of toxicity and mortality for the first 4 hours and subsequently for 24 hours. The LD_{50} (acute toxicity dose) was calculated using the following formula:

LD_{50} = √(highest non-lethal dose × least lethal dose)

All the animals used for the study were continually observed daily for two weeks (14 days) for delayed signs of toxicity and mortality.

Pentylenetetrazole-induced seizure

Pentylenetetrazole-induced seizure test was carried out using the method described previously by Yuskaitis *et al*. [20] Twenty-five Swiss albino mice of both sexes were used for the study. The mice were randomly assigned into two groups of five animals each. They were treated with vehicle (distilled water), extract or standard drug (diazepam). Group 1 (negative control) received distilled water 10 ml/kg, group 2 – 4 received *V. chrysocarpa* extract 200, 400 and 800 mg/kg p.o respectively, while group 5 was administered diazepam 0.5 mg/kg i.p. One hour after oral administration or 30 minutes after intraperitoneal administration, pentylentetrazole (80 mg/kg) was administered to each mouse intraperitoneally and was placed in an observation chamber. The onset of seizure and the survival time were recorded.

Elevated plus maze test

Elevated plus maze test was carried out using the method described previously by Carobrez *et al*. [21] Twenty-five Swiss albino mice of both sexes were used for the study. The mice were randomly assigned into five groups of five animals each. They were treated with vehicle, extract or standard drug. Group 1 (negative control) received distilled water 10 ml/kg, group 2 – 4 received *V. chrysocarpa* methanol extract 200, 400 and 800 mg/kg p.o respectively, while group 5 was administered diazepam 0.5 mg/kg i.p. One hour after oral administration or 30 minutes after intraperitoneal administration, each mouse was placed on the elevated plus maze apparatus and observed for 5 minutes. The number of entries to the respective arms and time spent were recorded.

Statistical analysis

The data obtained was expressed as mean standard error of mean (Mean ± SEM). One way analysis of variance (ANOVA) followed by Dunnet's post hoc test was used to test for significance $p < 0.05$ was considered significant. Graph pad prism (version 8.0) was used for the analysis.

Results and Discussion

Vitex chrysocarpa is an herbal plant with several ethnomedicinal applications. This study evaluated the antioxidant, anxiolytic and anti-epileptogenic potentials of its leaf methanol extract using established preclinical models. The study outcome revealed that the extract has potent antioxidant, anxiolytic and anti-epileptogenic potentials as demonstrated in the different models employed for the study.

Pharmacological activities of herbal medicines are a function of their bioactive principles. Hence, the importance of elucidating the chemicals present in herbal preparations suspected of having potent effects. Qualitative phytochemical analysis of *Vitex chrysocarpa* leaf methanol extract revealed the presence of important bioactive phyto-constituents including saponins, alkaloids, phenols, and flavonoids (Table 1).

Table 1: Qualitative phytochemical screening of the extract

Phytochemical	Inference
Tannins	+
Saponins	+
Alkaloids	+
Gylcosides	+
Steriods	+
Phenols	+
Flavanoids	+

+=Present

In the antioxidant study, the extract demonstrated good activity in the different models used to evaluate its antioxidant property. The extract had a high total antioxidant capacity and its ABTS inhibitory activity was 47.28 % at the highest dose used. It also had high total phenolic and total flavonoid contents (Table 2). Flavonoids and other phenolic compounds present in plants have been widely reported to elicit potent antioxidant activities in several experimental models [22 - 23] hence corroborates the observations from this study.

Result from the acute oral toxicity test showed that there was no mortality even at 5000 mg/kg. Reports by Corbet *et al.*,[24] Kennedy *et al*.,[25] and Syahmi *et al.*,[26] stated that test agents LD_{50} values more than 5000 mg/kg p.o are regarded as having high safety margin.

The elevated plus maze model is a commonly used test to evaluate for potential anxiolytic agents in rodents. Parameters such as number of entry and time spent in the respective arms (open and closed arms) are the indices for assessment. In this study, pre-treatment of the experimental animals with graded dose of the extract as well as the standard anxiolytic drug diazepam elicited anxiolytic-like behavior by increasing time spent in the open arm compared to the control, with (400 mg/kg) giving a better effect which was significant ($p < 0.05$) suggesting non-dose dependent effect (Figure 1 - 2).

Table 2: Antioxidant property of the extract

Conc. (µg/mL)	Total flavonoids content (mg rutin/g dw)	FRAP Assay (µM Fe (II)/g)	TAC (mg of GAE/g dw)	ABTS (% inhibition)	Total phenol content (mg GAE/g dw)
100	94.43 ± 0.887	28.48 ± 7.170	604.5 ± 13.16	25.28 ± 0.531	141.0 ± 7.422
200	107.9 ± 6.034	22.60 ± 6.449	513.6 ± 82.69	31.65 ± 0.236	145.2 ± 9.657
300	110.9 ± 1.578	24.03 ± 2.056	618.7 ± 14.49	39.91 ± 1.962	163.0 ± 7.988
400	116.7 ± 17.35	33.13 ± 1.891	626.5 ± 39.92	47.28 ± 5.063	165.1 ± 9.167

n = 3, Values expressed as Mean ± SEM

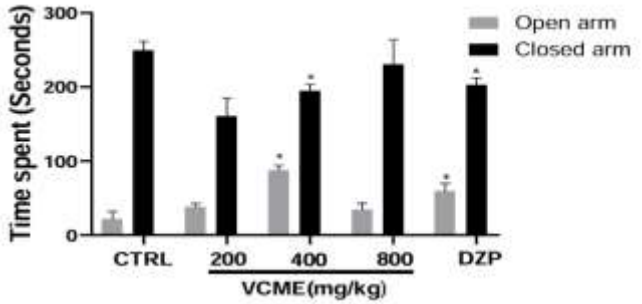

Figure 1: Anxiolytic effect of the extract in elevated plus maze test (Time spent in each arm).Values expressed as mean ± SEM, where n = 5, *significant at $p < 0.05$, using one way ANOVA and Dunnet's post-hoc test. VCME = *Vitex chrysocarpa* leaf methanol extract, DZP = diazepam, CTRL= Negative control (Water)

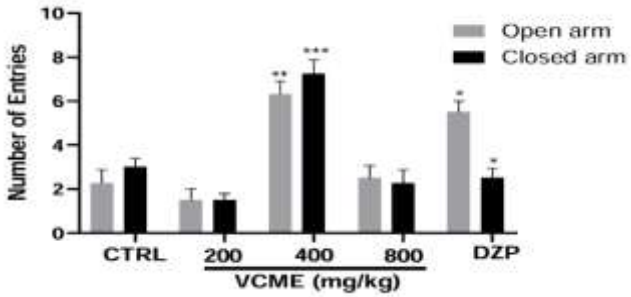

Figure 2: Anxiolytic effect of the extract in Elevated plus maze test (Number of entries) Values expressed as mean ± SEM, where n = 5, *significant at $p < 0.05$, using one way ANOVA and Dunnet's post-hoc test. VCME = *Vitex chrysocarpa* methanolic leaf extract, DZP = diazepam, CTRL= Negative control (Water)

Previous report by Phootha *et al*. [27] documented the anxiolytic effect of phenols, alkaloids and flavonoids and this lends credence to the study outcome. Reports from previous studies show that phytochemicals such as phenols, tannins and alkaloids elicit potent

anxiolytic effect which is mediated Gamma-aminobutyric acid (GABA), serotonin and adrenergic receptors. [1, 28] Hence, the anxiolytic effect observed during the study may have been mediated by any of these pathways.

Furthermore, in evaluating the anti-epileptogenic activity of the extract, the PTZ-induced seizure model was used. In this model, efficacious agents are expected to prolong seizure onset and prolong time of animal survival. Result from the study showed that the extract extended both seizure onset and the survival time when compared with the untreated animals. The extract demonstrated significant ($p < 0.05$) activity at 400 and 800 mg/kg compared to the untreated group (Figure 3). The observed activity may have been as a result of the inhibition of PTZ-induced excessive brain firing and this might be due to interaction with the GABA pathway or inhibition of neuronal oxidative damage.

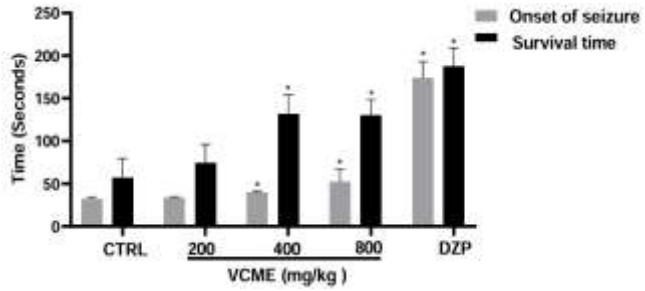

Figure 3: Anti-epileptogenic effect of the extract in pentylenetetrazole induced seizure. Values expressed as mean ± SEM, n = 5, *significant at $p < 0.05$ using ANOVA and Dunnet's post-hoc test. VCME = *Vitex chrysocarpa* leaf methanol extract, DZP = diazepam, CTRL= Negative control (Water)

Conclusion

The study outcome revealed that *V. chrysocarpa* leaf methanol extract demonstrated potent antioxidant, anti-epileptogenic and anxiolytic activities in the models used for the study. Hence may serve as scientific justification for its use in ethnomedicine and also provides useful information that may guide further mechanistic studies.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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